

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS

- 1 A method for detecting methylated nucleic acids comprising the steps of:
- (i) contacting a nucleic acid sample suspected of containing methylated nucleotides with an oligonucleotide sequence under suitable conditions for nucleic acid hybridization, said oligonucleotide sequence characterised in that,
- (a) it comprises a first stem labeled with a fluorophore moiety, a loop sequence having a region of nucleotides complementary to at least a region of the nucleic acid sample, which region is susceptible to methylation, and a second stem labeled with a quencher moiety that is capable of quenching the fluorophore moiety when in spatial proximity to the fluorophore moiety; and
- (b) the nucleotides forming the first stem are capable of moving into spatial proximity with the nucleotides forming the second stem when the probe is dissociated from the nucleic acid sample;
- (ii) altering the hybridization conditions such that the oligonucleotide probe dissociates from unmethylated DNA but remains hybridized to methylated DNA; and
- (iii) measuring the change in fluorescence
- 2 A method according to claim 1 wherein when the labeled oligonucleotide sequences dissociate from the target nucleic acid sample according to step (ii) the first and second stem hybridise together causing quenching of the fluorophore moiety.
- 3 A method according to claim 1 wherein the loop sequence contains at least about 10 nucleotides.
- 4 A method according to claim 1 wherein the loop sequence contains at least about up to 35 nucleotides.
- 5 A method according to claim 1 wherein the loop sequence contains at least about 25 nucleotides.

- 6 A method according to claim 1 wherein the loop sequence contains at least about from 15-20 nucleotides.
- 7 A method according to claim 1 wherein when the loop sequence is complementary to a portion of a nucleic acid sequence that undergoes methylation when a cell transforms from a normal state to a cancerous state.
- 8 A method according to claim 1 wherein when the loop sequence is complementary to a portion of a Myf-3 nucleic acid sequence that undergoes methylation when a cell transforms from a normal state to a cancerous state.
- 9 A method according to claim 8 wherein the labelled oligonucleotide sequence is complementary to at least one of the sequences selected from the group consisting of:
- 10 (i) 5' GCG GCG ACT CCG ACG CGT CCA GCC CGC GCT CC 3'
- (ii) 5' TTA TAC CGC AGG CGG GCG AGC CGC GGG CGC TCG CT 3'
- (iii) 5' CCG AGA GCC CTG CGG GGC CCG CCC TCC TGC TGG CG 3'
- 15 10 A method according to claim 1 wherein when the loop sequence is complementary to a portion of a glutathione-S-transferase-II(pi) nucleic acid sequence that undergoes methylation when a cell transforms from a normal state to a cancerous state.
- 11 A method according to claim 10 wherein the labelled oligonucleotide sequence is complementary to at least one of the sequences selected from the group consisting of:
- 20 i) 5' CTC CAG CGA AGG CCT CGC GGC CTC CGA GCC TTA TAA G 3'
- ii) 5' GGG GAC GCG GGC CGC GCG TAC TCA CTG GTG GCG A 3'
- 12 A method according to claim 1 wherein when the loop sequence is complementary to a portion of a calcitonin nucleic acid sequence that undergoes methylation when a cell transforms from a normal state to a cancerous state.
- 25 13 A method according to claim 1 wherein the method is used to detect abnormally methylated gene sequences in prostate cancer tissues.

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- 14 A method according to claim 1 wherein the hybridization condition that is altered during the hybridization reaction is the temperature of the hybridization reaction.
- 5 15 A method according to claim 1 wherein the stem sequences do not hybridise to the target gene and are of a sufficiently short length to avoid non-specific binding between the stem and any other nucleic acid sequence in the reaction mixture.
- 16 A method according to claim 1 wherein the stem sequences are at least about 4 to 8 nucleotides in length.
- 10 17 A method according to claim 1 wherein at least a cytosine in at least one of the stem sequences contains a methylated cytosine residue.
- 18 A kit comprising a labeled oligonucleotide sequence as described herein, which is adapted to distinguish methylated and non-methylated nucleic acid sequences when used in the method according to claim 1.
- 15 19 A method according to claim 1 substantially as herein before described.

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